

## OXYGEN DIFFUSION INTO CELLS

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Krogh (1919) determined the coefficients of diffusion of oxygen through surviving frog muscle and through fascia by using these as tissue membranes and measuring the gas diffusing through under given conditions. He applied the measured values in a formula relating capillary size and number to the oxygen needs of muscle *in vivo*.

These constants have been utilized by subsequent workers for substitution in other equations applying to the equilibrium state. Warburg (1923) developed an equation relating the necessary oxygen pressure to the metabolism and thickness of a slice of isolated tissue. Fenn (1927) and Gerard (1927) independently presented analogous equations for a cylinder of tissue, and A. V. Hill (1928) has extensively surveyed the general question of diffusion in tissues and developed several formulæ for the attaining of equilibrium as well as for the equilibrium state. Applying these equations to the specific question of diffusion of phosphate ion (Stella, 1928) and lactate ion (Eggleton, Eggleton and Hill, 1928), the values for the diffusion constants obtained suggested that these ions moved mainly in the intercellular fluid and with much greater difficulty across cell boundaries.

Should a similar situation obtain for oxygen, the calculations based on Krogh's constant might be considerably in error, and in any case, since this value has been so widely used, any further checks on its magnitude should be of value.

The simplest situation for studying oxygen penetration into living cells is offered, of course, by the unicellular organisms, which present, mainly, the problem of the sphere. It was the original intent of this work to evaluate the diffusion constant for cells as the only unknown in a simple equation, but it soon became apparent that a more searching examination of the assumptions made in such a simplified derivation was necessary. The elementary equation for diffusion into a sphere is easily derived as follows:

At equilibrium, the amount of oxygen diffusing into a mass of tissue must equal the amount consumed by it in the same time. For unit time, the inward diffusion depends on the surface area ( $4\pi r^2$  for the sphere); the diffusion coefficient,  $D$ ; and the gradient

of oxygen concentration along a radius,  $dC/dr$ ,  $C$  being the oxygen concentration at radius  $r$ . The consumption of oxygen by a sphere of indeterminate radius,  $r$ , is the product of the measured consumption per unit volume  $\left( = \frac{\text{mass}}{\text{sp. gr.}} = \text{mass} \times 0.95 \text{ for most protoplasm} \right)$ ,  $A$ , times the volume,  $\frac{4}{3} \pi r^3$ . At equilibrium, then

$$4\pi r^2 D \frac{dC}{dr} = \frac{4}{3} \pi r^3 A$$

or

$$\frac{dC}{dr} = \frac{Ar}{3D}; \quad (1)$$

which gives on integration

$$C = \frac{Ar^2}{6D} + K.$$

At  $r = 0$ ,  $C = K$  and  $K$  therefore represents the oxygen concentration at the center of the sphere.

In the case of most tissues so far studied, the oxygen consumption,  $A$ , has been found to be independent of the oxygen pressure over a wide range. The assumption has been made in this derivation that consumption in any one region is independent of oxygen concentration, provided this is greater than zero. Then for the critical condition where the oxygen concentration just reaches zero at the center of the sphere,  $K = 0$ , and the oxygen concentration at any level in the sphere is

$$C = \frac{Ar^2}{6D};$$

and at the surface,  $r = r_0$ , the critical concentration of oxygen needed to just insure a supply at the center is

$$C_0 = \frac{Ar_0^2}{6D} \quad (2)$$

and

$$D = \frac{Ar_0^2}{6C_0}. \quad (3)$$

$A$  may be fairly simply determined with one of the available methods of measuring oxygen consumption, and  $C_0$  is that concentration of oxygen in the medium which just gives full values of oxygen consumption. With lower concentrations the observed oxygen consumption begins to fall off. If the radius of the cell is known, a simple

means is available for obtaining the oxygen diffusion coefficient, or for bringing out the invalidity of assumptions as to the conditions of respiration.

It may be stated at once that the results of applying this simple equation to a variety of cells, as compared with results experimentally obtained, show such great variation as to indicate that other factors than the simple diffusion constants must be involved. It is necessary, then, to examine in some detail the assumptions made in the above derivation. Probably the most important is that just expressed, namely that the oxygen consumption of any region is independent of the concentration of oxygen at that region. This will be examined later. Other assumptions of little probable importance in the case of tissues may become extremely significant in the case of the individual cell. These are, first, that the oxygen consumption per individual region is a constant throughout the cell, that is, that the total consumption divided by the total volume gives the true consumption of each region. This is certainly not necessarily true, as the rate of respiration of a nuclear region or a cortical region might clearly be very much greater than that in other regions. It is also implicitly assumed that the diffusion constant, whether equal to that of Krogh or not, is at least constant throughout the cell. This also is not at all certain; in fact, in view of the previous discussion, it is entirely possible that the constant would vary widely across the membrane and in the cell interior.<sup>1</sup>

The mathematical development which follows is designed first to include all possible cases in which diffusion or consumption in two concentric regions of the cell are not assumed to be equal; and, following that, the more difficult case of the dependence of consumption on oxygen concentration will be considered. In this treatment I have called freely upon the expert assistance, generously given, of Dr. Walter Bartky of the Department of Astronomy at this University. The final section of the paper will apply the equations derived to the data available in the literature as well as to our own measurements, in an attempt to evaluate the conditions actually existing as regards respiration. Obviously, the derivations apply equally to other diffusible substances formed in and leaving or entering and utilized by the cell.

<sup>1</sup> The further complication, in many cells, of protoplasmic streaming cannot be handled rigorously. It is probably not significant in bacteria, yeast, or even *Arbacia* eggs; and when it is present, the stirring action would act as a decrease in radius in lowering the diffusion limit.

1. *Oxygen Consumption not Alike in All Portions of A Sphere*

a. *Limited to a central core (nuclear respiration).*—Let  $Q$  = the total oxygen consumed by the cell;  $C$  = the oxygen concentration at radius,  $r$ ;  $r_0$  = radius at cell surface;  $r'$  = radius of respiring region.

The oxygen diffusing into a sphere centered at the cell center and of any radius,  $r$ , equals  $4\pi r^2 D \frac{dC}{dr}$ . When  $r \geq r'$ , all oxygen consumed by the cell must pass through to the region of consumption, so:

$$4\pi r^2 D \frac{dC}{dr} = Q.$$

The consumption per unit volume in the non-respiring portion of the cell is zero; of the respiring portion:  $\frac{Q}{\frac{4}{3}\pi r'^3}$ .

When  $r \equiv r'$ , then, the oxygen passing the shell must equal the volume within this shell times the consumption per unit volume, or

$$4\pi r^2 D \frac{dC}{dr} = \frac{4}{3}\pi r'^3 \frac{Q}{\frac{4}{3}\pi r'^3}.$$

Then

$$\frac{dC}{dr} = \frac{Q}{4\pi r^2 D}, \quad \text{when } r \geq r', \quad (4a)$$

$$\frac{dC}{dr} = \frac{rQ}{r_0^3 4\pi D}, \quad \text{when } r \equiv r'. \quad (4b)$$

Integrating:

$$C = -\frac{Q}{4\pi D r} + K_1, \quad r \geq r', \quad (5a)$$

$$C = \frac{Qr^2}{8\pi D r_0^3} + K_2, \quad r \equiv r'. \quad (5b)$$

For the critical condition,  $C = 0$  when  $r = 0$  and  $C > 0$  for  $r > 0$ , then  $K_2 = 0$ ; and since  $C$  is continuous at  $r = r'$ ,

$$-\frac{Q}{4\pi D r'} + K_1 = \frac{Qr'^2}{8\pi D r'^3}$$

or

$$K_1 = \frac{3Q}{8\pi D r'}.$$

So that, from (5a),

$$C = \frac{Q}{4\pi D} \left( \frac{3}{2r'} - \frac{1}{r} \right), \quad r \geq r'.$$

At the cell surface  $r = r_0 \cong r'$ ,

$$C_c = \frac{Q}{4\pi D} \left( \frac{3}{2r'} - \frac{1}{r_0} \right). \quad (6)$$

( $C_c$  = oxygen concentration needed at the surface, radius =  $r_0$ , to just insure full penetration, in this case when all the oxygen is consumed within a sphere of radius  $r'$ .)

For the special case when  $r' = r_0$ , or oxygen consumption is uniform throughout the cell, this reduces to

$$C_0 = \frac{Q}{8\pi D r_0}$$

or, since  $A$  = consumption per unit volume,

$$A = \frac{Q}{\frac{4}{3}\pi r_0^3},$$

and therefore

$$C_0 = \frac{A r_0^2}{6D},$$

the original equation.

In general,

$$\frac{C_c}{C_0} = \frac{3 r_0}{r'} - 2. \quad (7)$$

It is readily seen from equation (7) that as  $r'$  decreases relative to  $r_0$ , the critical oxygen pressure needed,  $C_c$ , increases rapidly relative to  $C_0$ . When  $r' = \frac{1}{2}r_0$ ,  $C_c = 4C_0$ ; when  $r' = \frac{1}{4}r_0$ ,  $C_c = 10C_0$ .

*b. Oxygen consumption limited to an outer shell (cortical region).*—By an entirely analogous derivation it can be shown for this case that

$$\frac{C_c}{C_0} = \frac{(r_0 + 2r')(r_0 - r')}{r_0^2 + r_0 r' + r'^2}. \quad (8)$$

From this equation it appears that as the respiring shell of thickness  $(r_0 - r')$  thins,  $\frac{C_c}{C_0}$  decreases towards zero. When

$$r' = 0, \frac{C_c}{C_0} = 1; \quad r' = \frac{1}{2}r_0, \frac{C_c}{C_0} = 0.57; \quad r' = \frac{3}{4}r_0, \frac{C_c}{C_0} = 0.27;$$

$$r' = 0.9r_0, \quad \frac{C_c}{C_0} = 0.10.$$

*c. Oxygen consumption present throughout cell, but more or less intense in an outer shell.*—This will be considered in the general case below. See Equations 17 and 18.

## 2. *Diffusion Constant Different in Central and Cortical Parts of Cell*

If the diffusion constant is smaller at one region than another—as that of a relatively impermeable membrane is small compared to that of a permeable cell interior—the following derivation becomes necessary.

Let the diffusion coefficient be  $D'$  for the cell interior from  $r = 0$  to  $r = r'$ ; and  $D$  for a superficial shell,  $r = r'$  to  $r = r_0$ . Then, by a familiar derivation,

$$\begin{aligned} \text{When } r \leq r', \quad 4\pi r^2 D' \frac{dC}{dr} &= \frac{4}{3} \pi r^3 A; & \frac{dC}{dr} &= \frac{Ar}{3D'} \\ \text{When } r \geq r', \quad 4\pi r^2 D \frac{dC}{dr} &= \frac{4}{3} \pi r^3 A; & \frac{dC}{dr} &= \frac{Ar}{3D}. \end{aligned}$$

Integrating between limits, and assuming  $C = 0$  for  $r = 0$ , and  $C > 0$  for  $r > 0$ ,

$$C' = \frac{Ar'^2}{6D'},$$

$$C_0 - C' = \frac{A(r_0^2 - r'^2)}{6D},$$

where  $C'$  is the concentration at  $r'$ . Combining,

$$C_0 = \frac{A}{6} \left( \frac{r_0^2 - r'^2}{D} + \frac{r'^2}{D'} \right), \quad (9)$$

which reduces to the simple equation (2) when  $D = D'$  or  $r' = r_0$ .

It is obvious from (8) that if the cell membrane is thin compared to the cell radius, that is,  $r'$  is nearly as large as  $r_0$ , its diffusion constant,  $D$ , must be very small in relation to that of the interior,  $D'$ , to affect  $C_0$ . For example, to double  $C_0$  when  $r' = 0.9r_0$ ,  $D$  must be  $0.16D'$ ; and when  $r'$  is  $0.99r_0$ ,  $D = 0.02D'$ . To increase  $C_0$  ten times: for  $r' = 0.9r_0$ ,  $D = 0.012D'$ ; for  $r' = 0.99r_0$ ,  $D = 0.002D'$ . Since it appears from the work of the Eggletons and Hill (1928) that the diffusion coefficient for ions across the cell surface may be one hundredth as large as in the cell or intercellular fluid, this factor obviously might be of importance for oxygen diffusion. But unionized molecules appear to penetrate membranes much more easily than do ions, so that the analogy may not hold.

In all the above derivations the critical oxygen pressure at the cell surface has been calculated. That is, assuming that the oxygen consumption of the whole cell remains at its maximum value, what is the necessary external pressure as the various conditions within the

cell are changed? It is next desirable to investigate the oxygen consumption-external oxygen pressure curve below this critical value and to examine its shape for the simplest situation.

Let  $r_1$  represent the depth of oxygen penetration, *i.e.*,  $C = 0$  for values of  $r$  such that  $r_1 \geq r \geq 0$ , and  $C > 0$  for  $r > r_1$ . And let  $C_s$  represent the oxygen concentration at the surface,  $r = r_0$ .

Then, as the respiring volume  $= \frac{4}{3} \pi (r^3 - r_1^3)$ ,

$$\begin{aligned} 4\pi r^2 D \frac{dC}{dr} &= \frac{4}{3} \pi A (r^3 - r_1^3), \\ \frac{dC}{dr} &= \frac{A(r^3 - r_1^3)}{3Dr^2}, \\ C_s &= \frac{A}{3D} \int_{r_1}^{r_0} \frac{r^3 - r_1^3}{r^2} dr, \\ &= \frac{A}{3D} \left( \frac{r_0^2}{2} + \frac{r_1^3}{r_0} - \frac{3}{2} r_1^2 \right). \end{aligned} \quad (10)$$

Similarly, the oxygen consumed is:

$$\begin{aligned} Q &= 4\pi \int_{r_1}^{r_0} A r^2 dr, \\ Q &= \frac{4\pi A}{3} (r_0^3 - r_1^3). \end{aligned} \quad (11)$$

Take units such that  $r_0 = 1$ , and that  $C_s = C_0 = 1$  for  $r_1 = 0$ , and also that  $Q = 1 = Q_0$  for  $r_1 = 0$ . Note that with this selection of units,  $\frac{A}{D} = 6$ ,  $A = \frac{3}{4\pi}$ ,  $D = \frac{1}{8\pi}$ . The relation between  $Q$  and  $C$  for the general case is then expressed in terms of  $r$  as a parameter: From (10)

$$C_s = 1 - r_1^2(3 - 2r_1),$$

and from (11)

$$Q = 1 - r_1^3,$$

which give the following values (Table I). The corresponding curve is given in Fig. 1.

Still more generally, assuming only that  $A$  is constant as regards  $C$ , the following derivation includes all cases involving a single concentric zoning of the cell as regards respiration and diffusion.

Let  $r'$  represent the surface separating two cell zones.

Let  $D'$  and  $A'$  represent the diffusion coefficient and unit oxygen consumption for the nuclear region,  $r < r'$ ; and similarly  $D$  and  $A$  represent these values for the cortical region,  $r > r'$ .

TABLE I

| $\frac{C_s}{C_0}$ | $\frac{r_1}{r_0}$ | $\frac{Q}{Q_0}$ |
|-------------------|-------------------|-----------------|
| 1.00              | 0.0               | 1.00            |
| 0.90              | 0.2               | 0.99            |
| 0.65              | 0.4               | 0.94            |
| 0.50              | 0.5               | 0.87            |
| 0.35              | 0.6               | 0.78            |
| 0.22              | 0.7               | 0.66            |
| 0.10              | 0.8               | 0.49            |
| 0.04              | 0.9               | 0.27            |
| 0.00              | 1.0               | 0.00            |

Let  $r_1$  represent, as previously, the depth of oxygen penetration. In the following it is assumed that  $r_1 \leq r'$ , for when  $r_1 > r'$  the situation reduces to the simple cases previously considered, inasmuch as no oxygen reaches the inner sphere where conditions are different.

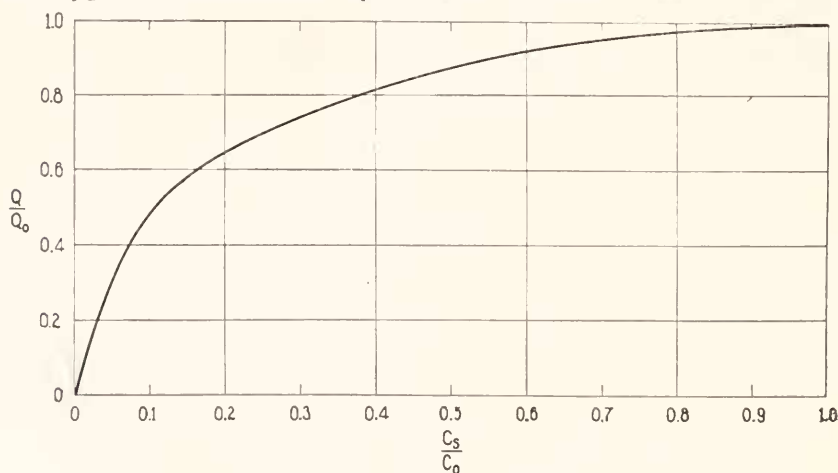


FIG. 1.

Let  $C'$  represent the oxygen concentration at  $r = r'$ ;  $C_s$  the oxygen concentration at the cell surface,  $r = r_0$ ; and  $C_c$  the critical surface concentration to just insure complete penetration. For the simple case,  $C_c = C_0$ .

Then the derivation, along the previous lines, is:

$$\text{For } r < r', \quad 4\pi r^2 D' \frac{dC}{dr} = \frac{4}{3} \pi A' (r^3 - r_1^3); \quad (12a)$$

$$r > r', \quad 4\pi r^2 D \frac{dC}{dr} = \frac{4}{3} \pi (r'^3 - r_1^3) A' + \frac{4}{3} \pi A (r^3 - r'^3). \quad (12b)$$



Simplifying:

$$\text{For } r < r', \quad \frac{dC}{dr} = \frac{A'}{3D'} \frac{(r^3 - r_1^3)}{r^2},$$

and integrating from  $r_1$  to  $r'$ :

$$C' = \frac{A'}{3D'} \left( \frac{r'^2}{2} + \frac{r_1^3}{r'} - \frac{3r_1^2}{2} \right). \quad (13)$$

Similarly:

$$\text{For } r > r', \quad \frac{dC}{dr} = \frac{A'(r'^3 - r_1^3)}{3Dr^2} + \frac{A(r^3 - r'^3)}{3Dr^2}$$

and integrating from  $r'$  to  $r_0$ :

$$C_s - C' = \frac{A'(r'^3 - r_1^3)}{3D} \left( \frac{1}{r'} - \frac{1}{r_0} \right) + \frac{A}{3D} \left( \frac{r_0^2}{2} - \frac{r'^3}{r_0} - \frac{3}{2} r'^2 \right). \quad (14)$$

From (13) and (14)

$$C_s = \frac{A'(r'^3 - r_1^3)}{3D} \left( \frac{1}{r'} - \frac{1}{r_0} \right) + \frac{A}{3D} \left( \frac{r_0^2}{2} + \frac{r'^3}{r_0} - \frac{3}{2} r'^2 \right) + \frac{A'}{3D'} \left( \frac{r'^2}{2} + \frac{r_1^3}{r'} - \frac{3}{2} r_1^2 \right). \quad (15)$$

Equation (15) may be explored as follows:

For  $r_1 = 0$ , that is, at the critical external oxygen pressure,

$$C_c = \frac{A'r'^3}{3D} \left( \frac{1}{r'} - \frac{1}{r_0} \right) + \frac{A}{3D} \left( \frac{r_0^2}{2} + \frac{r'^3}{r_0} - \frac{3}{2} r'^2 \right) + \frac{A'r'^2}{6D'}. \quad (16)$$

For  $r_1 = r' = 0$

$$C_c = \frac{Ar_0^2}{6D}.$$

For  $r_1 = 0$ ,  $r' = r_0$

$$C_0 = \frac{A'r_0^2}{6D'}.$$

For  $r_1 = r'$

$$C_s = \frac{A}{6D} \left( r_0^2 + \frac{2r'^2}{r_0} - 3r'^2 \right). \quad (\text{See Equation 10.})$$

For  $r_1 = r' = r_0$

$$C_s = 0.$$

For  $D = D'$

$$\begin{aligned} C_s &= \frac{A'}{3D} \left[ \frac{3}{2} (r'^2 - r_1^2) - \frac{r'^3 - r_1^3}{r_0} \right] + \frac{A}{3D} \left( \frac{r_0^2}{2} + \frac{r'^3}{r_0} - \frac{3}{2} r'^2 \right) \\ &= \frac{(A - A')r'^2}{6D} \left( \frac{2r'}{r_0} - 3 \right) + \frac{Ar_0^2}{6D} + \frac{A'r_1^2}{6D} \left( \frac{2r_1}{r_0} - 3 \right). \end{aligned} \quad (17)$$

If  $A = A'$ , or  $r_1 = r'$ ,

$$\text{For } r_1 = 0, \quad C_s = \frac{A}{6D} \left( r_0^2 + \frac{2r_1^3}{r_0} - 3r_1^2 \right). \quad (\text{See Equation 10.})$$

$$C_c = \left( \frac{A - A'}{6D} \right) r'^2 \left( \frac{2r'}{r_0} - 3 \right) + \frac{A r_0^2}{6D}. \quad (18)$$

If  $A = A'$ ,

$$C_0 = \frac{A r_0^2}{6D}.$$

For  $A = A'$

$$\begin{aligned} C_s &= \frac{A}{3D} \left[ \frac{r_0^2 - r'^2}{2} - r_1^3 \left( \frac{1}{r'} - \frac{1}{r_0} \right) \right] + \frac{A}{3D'} \left( \frac{r'^2}{2} + \frac{r_1^3}{r'} - \frac{3}{2} r_1^2 \right) \\ &= \frac{A}{6} \left( \frac{1}{D'} - \frac{1}{D} \right) \left( r'^2 + \frac{2r_1^3}{r'} \right) + \frac{A}{6} \left( \frac{r_0^2 - \frac{2r_1^3}{r_0}}{D} - \frac{3r_1^2}{D'} \right). \end{aligned} \quad (19)$$

If  $D = D'$  or  $r_1 = r'$ ,

$$\text{For } r_1 = 0, \quad C_s = \frac{A}{6D} \left( r_0^2 - \frac{2r_1^3}{r_0} - 3r_1^2 \right) \quad (\text{See Equation 10.})$$

$$\begin{aligned} C_c &= \frac{A}{3D} \left( \frac{r_0^2 - r'^2}{2} \right) + \frac{A r'^2}{6D'} \\ &= \frac{A}{6} \left( \frac{r_0^2 - r'^2}{D} + \frac{r'^2}{D'} \right). \quad (\text{See Equation 9.}) \end{aligned}$$

If  $D = D'$ ,

$$C_0 = \frac{A r_0^2}{6D}.$$

The expression for oxygen consumed per cell is:

$$\begin{aligned} Q &= 4\pi A' \int_{r_1}^{r'} r^2 dr + 4\pi A \int_{r'}^{r_0} r^2 dr \\ &= \frac{4}{3} \pi [A'(r'^3 - r_1^3) + A(r_0^3 - r'^3)]. \end{aligned} \quad (20)$$

Table II and Fig. 2 give the  $Q - C_s$  curves obtained for various relationships of  $\frac{r'}{r_0}$ ,  $\frac{D}{D'}$ ,  $\frac{A}{A'}$ .

Consider finally the general case where  $A \propto F(C)$  and is not always a constant, though approximately so for high values of  $C_0$ . The amount of oxygen crossing a spherical surface of radius  $r$  is  $4\pi r^2 D \frac{dC}{dr}$ ;

therefore that consumed in a shell of internal radius =  $r$ , external radius =  $r + dr$ , is

$$4\pi \left( r^2 D \frac{dC}{dr} \right) \Big|_{r+dr} - 4\pi \left( r^2 D \frac{dC}{dr} \right) \Big|_r.$$

This is equal to the consumption per unit volume times the volume:  $4\pi r^2 dr$ .

$A$  is some function of  $C = DF(C)$ , where  $F(C)$  does not decrease with increase in  $C$ , i.e., a monotonically increasing function of  $C$ . Then

$$\left( r^2 D \frac{dC}{dr} \right) \Big|_{r+dr} - \left( r^2 D \frac{dC}{dr} \right) \Big|_r = DF(C) r^2 dr$$

or

$$\frac{d \left( r^2 D \frac{dC}{dr} \right)}{dr} = r^2 DF(C);$$

and, assuming  $D$  independent of  $C$  and  $r$ ,

$$\frac{d \left( r^2 \frac{dC}{dr} \right)}{dr} = r^2 F(C). \quad (21)$$

The total oxygen consumption of a cell is

$$\begin{aligned} Q &= \int_0^{r_0} 4\pi r^2 dr \\ &= 4\pi D \int_0^{r_0} F(C) r^2 dr. \end{aligned} \quad (22)$$

$Q$  as a function of  $C_0$  is an experimentally observable relationship, from which it is desired to obtain  $F(C)$ . Consider the most general case, that is, either  $C = 0$  for  $r = r_1$ , where  $r_1 \geq 0$ , or  $C > 0$  for all values of  $r$ . For this latter, always take  $r_1 = 0$  in what follows. Integrating (21) between  $r_1$  and  $r$ ,

$$\begin{aligned} r^2 \frac{dC}{dr} &= \int_{r_1}^r r^2 F(C) dr, \\ \frac{dC}{dr} &= \frac{1}{r^2} \int_{r_1}^r r^2 F(C) dr; \end{aligned}$$

and integrating between  $r$  and  $r_0$ ,

$$C_0 - C = \int_r^{r_0} \frac{1}{r^2} \left( \int_{r_1}^r r^2 F(C) dr \right) dr,$$

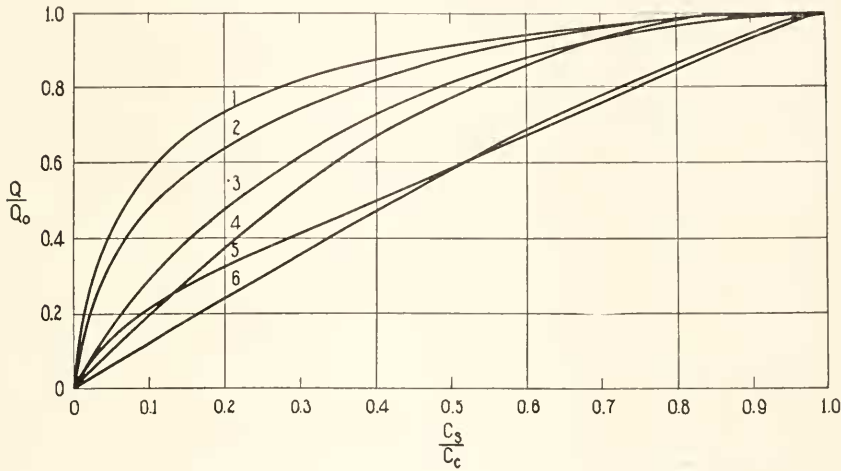
or

$$C = C_0 - \int_r^{r_0} \frac{1}{r^2} \left( \int_{r_1}^r r^2 F(C) dr \right) dr. \quad (23)$$

TABLE II

| For $A = A'$                   |                                 |                                 |                                  |                                  |                                  |
|--------------------------------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|
| $C_s C_c$                      |                                 |                                 |                                  |                                  |                                  |
| $\frac{Q}{Q_b}$                | $r' = 0.9 r_0$<br>$D = 0.01 D'$ | $r' = 0.98 r_0$<br>$D = 0.1 D'$ | $r' = 0.67 r_0$<br>$D = 0.01 D'$ | $r' = 0.98 r_0$<br>$D = 0.1 D'$  | $r' = 0.9 r_0$<br>$D = 0.01 D'$  |
|                                |                                 |                                 |                                  |                                  |                                  |
| 1.00<br>(1.00)                 | 1.00<br>(1.37)                  | 1.00<br>(56.1)                  | 1.00<br>(1.00)                   | 1.00<br>(5.03)                   | 1.00<br>(2.08)                   |
| 0.99                           | 0.97                            | 0.98                            | 0.90                             | 0.96                             | 1.00                             |
| 0.94                           | 0.91                            | 0.88                            | 0.65                             | 0.86                             | 0.99                             |
| 0.875                          | 0.85                            | 0.77                            | 0.50                             | 0.80                             | 0.97                             |
| 0.79                           | 0.73                            | 0.61                            | 0.35                             | 0.69                             | 0.90                             |
| 0.66                           | 0.58                            | 0.33                            | 0.22                             | 0.56                             | 0.94                             |
| 0.49                           | 0.39                            | 0.18                            | 0.10                             | 0.40                             | 0.83                             |
| 0.27                           | 0.15                            | 0.09                            | 0.03                             | 0.21                             | 0.84                             |
| 0.14                           | 0.05                            | 0.04                            | 0.01                             | 0.11                             | 0.76                             |
|                                | Fig. 2,<br>Curve 5              | Curve 3                         | Curve 2                          |                                  | Fig. 2,<br>Curve 1               |
| $A = 20 A'$                    |                                 |                                 |                                  |                                  |                                  |
| $r' = 0.9 r_0$<br>$D = 0.1 D'$ | $r' = 0.98 r_0$<br>$D = 0.1 D'$ | $r' = 0.5 r_0$<br>$D = D'$      | $r' = 0.9 r_0$<br>$D = 0.1 D'$   | $r' = 0.98 r_0$<br>$D = 0.01 D'$ | $r' = 0.98 r_0$<br>$D = 0.01 D'$ |
|                                |                                 |                                 |                                  |                                  |                                  |
| $\frac{Q}{Q_b}$                | $\frac{C_s}{C_c}$               | $\frac{C_s}{C_c}$               | $\frac{Q}{Q_b}$                  | $\frac{Q}{Q_b}$                  | $\frac{C_s}{C_c}$                |
| 1.00<br>(6.13)                 | 1.00<br>(8.00)                  | 1.00<br>(1.76)                  | 1.00<br>(0.3)                    | 1.00<br>(0.79)                   | 1.00<br>(0.96)                   |
| 1.00                           | 1.00                            | 0.95                            | 0.95                             | 0.99                             | 0.99                             |
| 0.99                           | 0.94                            | 0.80                            | 0.79                             | 0.92                             | 0.93                             |
| 0.98                           | 0.91                            | 0.70                            | 0.58                             | 0.84                             | 0.86                             |
| 0.97                           | 0.87                            | 0.60                            |                                  | 0.73                             | 0.77                             |
| 0.95                           | 0.82                            | 0.50                            |                                  | 0.56                             | 0.64                             |
| 0.92                           | 0.76                            | 0.40                            |                                  | 0.35                             | 0.46                             |
|                                | 0.65                            | 0.31                            |                                  |                                  | 0.23                             |
|                                | 0.59                            | 0.27                            |                                  |                                  | 0.10                             |
|                                |                                 |                                 | Curve 4                          |                                  | Curve 6                          |

$C_s$  is the largest value of  $C$  and hence  $F(C_s)$  is the largest value  $F(C)$  can have. Replacing  $F(C)$  by this constant maximum value and also, in the case  $r \neq 0$ , replacing  $r$  by 0 (this gives a larger range



| Curve | $\frac{A}{A'}$ | $\frac{D}{D'}$ | $\frac{r'}{r_0}$ |
|-------|----------------|----------------|------------------|
| 1     | 5.0            | 1.00           | 0.67             |
| 2     | 1.0            | 1.00           | 1.00             |
| 3     | 1.0            | 0.10           | 0.98             |
| 4     | 0.2            | 0.10           | 0.90             |
| 5     | 1.0            | 0.01           | 0.90             |
| 6     | 0.2            | 0.01           | 0.98             |

See text for details.

FIG. 2.

of integration, so that a larger value of the integral is obtained), we have

$$C \cong C_s - \int_r^{\infty} \frac{1}{r^2} \left( \int_0^r r'^2 F(C_s) dr' \right) dr.$$

And, completing the integration,

$$C \cong C_s - \frac{F(C_s)}{6} (r_0^2 - r^2). \quad (24)$$

Hence, if  $F(C_s)$  is such that

$$F(C_s) < \frac{6C_s}{r_0^2}$$

$C$  is greater than 0 for any  $r$ . That is, if  $F(C)$  is such a function that

$$F(C_s) < \frac{6C_s}{r_0^2}$$

for a certain range of values of  $C_s$ , then in this range  $C$  is greater than 0 for  $r \geq 0$  for any  $C_s$ . In the further development this inequality will be assumed for the range of  $C_s$  employed. A first approximation may be obtained by assuming  $F(C) \ll \frac{6C}{r_0^2}$ . (See later.) Then from (24)  $C$  is approximately equal to  $C_s$ . And, as a second approximation,

$$\begin{aligned} C &= C_s - \int_r^{r_0} \frac{1}{r^2} \left( \int_0^r r^2 F(C_s) dr \right) dr \\ &= C_s - \frac{F(C_s)}{6} (r_0^2 - r^2). \end{aligned}$$

Returning to equation (22), the first approximation gives

$$\begin{aligned} Q &= 4\pi D \int_0^{r_0} F(C_s) r^2 dr \\ &= \frac{4\pi D F(C_s) r_0^3}{3}. \end{aligned}$$

That is, for high  $C_s$ ,  $Q = kF(C_s)$ , where  $k = \frac{4\pi D r_0^3}{3}$ . Using the second approximation,

$$Q = 4\pi D \int_0^r F \left[ C_0 - \frac{F(C_s)}{6} (r_0^2 - r^2) \right] r^2 dr.$$

Expanding, assuming  $C_s$  large compared with  $\frac{F(C_s)}{6} (r_0^2 - r^2)$ ,

$$\begin{aligned} Q &= 4\pi D \int_0^{r_0} \left[ F(C_s) - \frac{F(C_s)}{6} (r_0^2 - r^2) \frac{dF(C_s)}{dC_s} \right] r^2 dr \\ &= \frac{4\pi D r_0^3 F(C_s)}{3} \left[ 1 - \frac{r_0^2}{15} \frac{dF(C_s)}{dC_s} \right]. \end{aligned} \quad (25)$$

This shows that the effect of diffusion (in lowering  $C$  inside the cell and therefore  $A$  and  $Q$ ), represented by the term in brackets, is very small unless  $\frac{dF(C_s)}{dC_s}$  is very large.

Experimentally,  $Q$  is obtained as a function of  $C_s$ . Dropping the subscript,  $s$ ,

$$Q(C) = kF(C) \left[ 1 - \frac{r_0^2}{15} \frac{dF(C)}{dC} \right]. \quad (25a)$$

Let  $F(C) = \frac{Q(C)}{k} + r_0^2 H(C) + \text{higher powers of } r_0 \text{ times functions of}$

C. Substituting in (25a)

$$Q(C) = Q(C) + kr_0^2 H(C) - Q(C) \frac{r_0^2}{15k} \frac{dQ(C)}{dC}$$

plus higher powers of  $r_0$  times functions of  $C$ , or

$$H(C) = \frac{Q(C)}{15k^2} \frac{dQ(C)}{dC}.$$

Hence

$$\begin{aligned} F(C) &= \frac{Q(C)}{k} + \frac{r_0^2 Q(C)}{15k^2} \frac{dQ(C)}{dC} + \dots \\ &= \frac{3Q(C)}{4\pi Dr_0^3} \left( 1 + \frac{1}{20\pi Dr_0} \frac{dQ(C)}{dC} \right) + \dots \end{aligned}$$

By Taylor's theorem, neglecting higher powers,

$$F(C) = \frac{Q(C^*)}{k},$$

where

$$C^* = C + \frac{r_0^2}{15k} Q(C);$$

and since

$$A(C) = DF(C),$$

therefore

$$A(C) = \frac{3}{4\pi r_0^3} Q(C^*) \quad (26)$$

and

$$C^* = C + \frac{Q(C)}{20\pi Dr_0}. \quad (27)$$

It will be noted that the larger the value of  $D$ , the more nearly do  $Q(C)$  and  $Q(C^*)$  agree.  $Q$  decreases with decrease of  $C$  more rapidly than does the true function  $A(C)$ . This is due to the factor of diffusion, making  $C$  at interior regions of the cell lower than  $C_s$ . Obviously the closer  $C$  approaches  $C_s$ , the more nearly will the observed oxygen consumption of the whole cell equal the theoretical consumption per unit volume where this volume has an oxygen pressure of  $C_s$  throughout. The higher the diffusion constant and the smaller the radius, the closer will the approach be. The effect of  $D$  is apparent in equations (26) and (27), but an increase of radius,  $r_0$ , might at first seem to likewise reduce the diffusion factor. When it is recalled, however, that  $Q(C)$ , the total consumption of a cell, varies with  $r_0^3$ , it is clear that the diffusion effect increases as the square of the radius.

For unfertilized sea urchin eggs, Tang (1931) has obtained the data necessary for the calculations. These indicate that the maximal

correction is 0.5 per cent, if the diffusion constant be as assumed. For a constant 100 times smaller, the correction is important, and this case is illustrated in Table III and the resultant  $A(C)$  curve

TABLE III

For unfertilized *Arbacia* eggs:

$Q_0 = 6.7 \times 10^{-10}$  cc.  $O_2$  per minute, per egg.

$D = 1.1 \times 10^{-7}$  cc.  $O_2$  per minute, per  $cm.^2$  per diffusion gradient of one atmosphere per cm. (1/100 Krogh's value assumed).

$C_c = 0.1$  atmosphere.

$r_0 = 3.8 \times 10^{-3}$  cm.

| 1     | 2                 | 3                                | 4                    | 5     | 6                                | 7                    | 8                             |
|-------|-------------------|----------------------------------|----------------------|-------|----------------------------------|----------------------|-------------------------------|
| $C_s$ | $\frac{C_s}{C_c}$ | $\frac{Q(C_s)}{\times 10^{-10}}$ | $\frac{Q(C_s)}{Q_0}$ | $C^*$ | $\frac{Q(C^*)}{\times 10^{-10}}$ | $\frac{Q(C^*)}{Q_0}$ | $\frac{A(C)}{\times 10^{-3}}$ |
| 0.100 | 1.00              | 6.7                              | 1.00                 | 0.125 | 6.7                              | 1.00                 | 2.92                          |
| 0.090 | 0.90              | 6.6 +                            | 0.99                 | 0.115 | 6.7                              | 1.00                 | 2.92                          |
| 0.065 | 0.65              | 6.6 -                            | 0.98                 | 0.090 | 6.6 +                            | 0.99                 | 2.89                          |
| 0.050 | 0.50              | 6.4                              | 0.96                 | 0.074 | 6.6                              | 0.98                 | 2.86                          |
| 0.035 | 0.35              | 6.2                              | 0.92                 | 0.057 | 6.5                              | 0.97                 | 2.83                          |
| 0.022 | 0.22              | 5.7                              | 0.85                 | 0.044 | 6.3                              | 0.94                 | 2.74                          |
| 0.010 | 0.10              | 4.4                              | 0.66                 | 0.027 | 5.9                              | 0.88                 | 2.57                          |
| 0.004 | 0.04              | 3.1                              | 0.46                 | 0.016 | 5.0                              | 0.75                 | 2.19                          |
| 0.000 | 0.00              | 0.0                              | 0.00                 | 0.000 | 0.0                              | 0.00                 | 0.00                          |

shown in Fig. 3. For luminous bacteria, because of the very small  $r_0$ , the correction turns out to be entirely negligible and the  $Q(C)$  curve as observed is also the  $A(C)$  curve (Fig. 3).

Columns 1 and 3 are taken from the data on *Arbacia* eggs. These

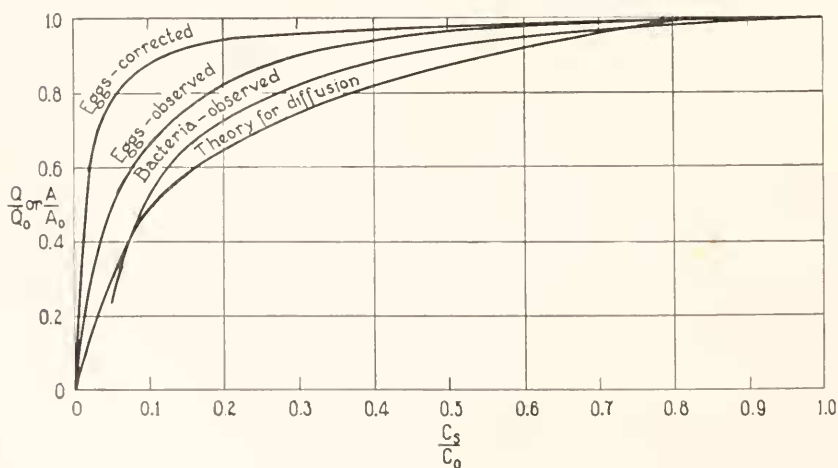


FIG. 3.



values are expressed as ratios in Columns 2 and 4.  $C^*$  is calculated by equation (27).  $Q(C^*)$  is again obtained by interpolation on the  $Q(C)$  curve for eggs, taking  $Q$  for each value of  $C^*$  as if of  $C$ . Column 7 is obtained from 6 and Column 8 from 6 and equation (26).  $A$ , as a function of  $C$ , is expressed as volume of oxygen per equal volume of eggs per minute.

It may be observed that the assumption made in this last derivation, that  $A \ll \frac{6CD}{r_0^2}$  is fully justified for this case. Table IV shows the actual values of each side of the inequality for several values of  $C$ .

TABLE IV

| $C$   | $A$    | $\frac{6CD}{r_0^2}$ |
|-------|--------|---------------------|
| 0.1   | 0.0029 | 0.480               |
| 0.05  | 0.0029 | 0.240               |
| 0.01  | 0.0026 | 0.048               |
| 0.004 | 0.0022 | 0.019               |

#### *Examination of Available Data*

There are a number of data in the literature for several types of cells sufficiently complete to permit the calculation of the simple  $C_0$ ; fewer giving as well the observed  $C_0$ . Table V summarizes most of this material. It will be noted that the observed  $C_0$  is from 30 to 1000 times greater than the calculated one in all cases except that reported by Warburg and Kubowitz (1929). These workers offer evidence that inadequate shaking at the low oxygen pressures may cause the liquid phase to lag behind the gas phase in oxygen pressure, the dissolved oxygen being used by the cells faster than it can be replaced from the gas phase. Where such a non-equilibrium state obtains, the actual  $C_0$  would be less than supposed, which might account for a reported high  $C_0$ . In the experiments of Gerard and Falk (1931), as well as those of Tang (1931), at least, this factor was considered and apparently excluded by controls of the rate of shaking. As pointed out by these workers, wide variation in the conditions of shaking did not affect the  $C_0$ . In Amberson's experiments no gas phase was present, only dissolved oxygen being available to the cells from a large volume of liquid. Also, it may be noted that Stephenson and Whetham (1924) found *B. coli* forming lactic acid in air but not in oxygen; and Novy and Soule (1925) observed for the case of tubercle bacilli that growth on the surface of an agar slant was retarded at

TABLE V

| Material                           | Observer                       | Radius cm.<br>$\times 10^{-4}$ | Temp.<br>° C. | Oxygen consumption<br>cu. mm. per minute   |   | $C_0$ (Atmosphere) |  |
|------------------------------------|--------------------------------|--------------------------------|---------------|--|---|--------------------|--|
|                                    |                                |                                |               | per $10^6$                                 | per cu. mm.                             | Observed           | Calculated *                                 |
| <i>Sarcina lutea</i> .....         | Gerard and Falk, 1931          | 0.63                           | 20            | $5 \times 10^{-6}$<br>$1.7 \times 10^{-4}$ | 0.05 (H <sub>2</sub> O)<br>0.17 (gluc.) | 0.01<br>0.025      | $1.2 \times 10^{-5}$<br>$3.8 \times 10^{-5}$ |
| Sea urchin eggs.....               | Tang, 1931<br>Amberson, † 1928 | 38                             | 21<br>20      | 0.67<br>3.3                                | 0.004 (unfert.)<br>0.022 (fert.)        | 0.10<br>0.10       | $7.4 \times 10^{-4}$<br>$2.7 \times 10^{-3}$ |
| Luminous bacteria.....             | Shoup, 1929<br>Harvey, 1928    | $0.6 \times 1.1$               | 21            | $5 \times 10^{-4}$                         | 0.3                                     | 0.03               | $1.9 \times 10^{-5}$                         |
| <i>Micrococcus candidans</i> ..... | Warburg and Kubowitz,<br>1929  | 0.4                            | 10            |  |   | 0.00005            | $\alpha 10^{-5}$                             |
| Yeast.....                         | Warburg, 1926                  | 3.5                            | 20            |  | 0.2 (gluc. and PO <sub>4</sub> )        | $0.04 < .20$       | $4 \times 10^{-6}$                           |
| <i>Amoeba</i> .....                | Pantin, 1930                   | $50 \times 13$                 | 15            |  |   | 0.05               | $5 \times 10^{-4} \dagger$                   |
| Nitrifying bacteria.....           | Meyerhof, 1916                 | .                              | 35            |  |   | 0.20               |  |
| <i>Colpidium</i> .....             | Pitts, (unpublished)           | $25 \times 15$                 | 24            | 2.5  | 0.1                                     |                    | $6 \times 10^{-3}$                           |

\*  $D$  has been taken throughout as equal to  $1.1 \times 10^{-5}$  (expressed as cc. O<sub>2</sub> diffusing per minute, per cm.<sup>2</sup> surface per gradient of 1 atmosphere per cm.)

† For fertilized *Arbacia* eggs, Amberson (1928) finds  $C_0 = 0.10$  atmospheres.  $A$  cannot be obtained from his data, but he informs me that 3 cc. of a settled egg suspension consumed 0.25 cc. O<sub>2</sub> per hour. If the eggs were just in contact, this would correspond to about 1.5 gm. of eggs, and  $A = 0.003$  cu. mm. O<sub>2</sub> per cu. mm. eggs per minute. This value is lower than that obtained by Tang by direct measurement for unfertilized *Arbacia* eggs, and less than 1/7 the corresponding one for fertilized eggs. A similar value for  $A$ , 0.002, can be calculated from Warburg's data (1908) on *Strongylocentrotus* eggs, if the same cell radius as *Arbacia* and a specific gravity of 1.05 be assumed.

All agree that fertilized eggs respire five to six times as rapidly as resting ones and the figure used here is 5.5 times that of Tang for the resting cells.

‡ Pantin's calculation.

oxygen pressures below 0.06 atmospheres. In Meyerhof's experiments (1916) also, vigorous shaking is mentioned, and Warburg (1926) found the oxygen consumption of yeast to fall with oxygen pressures below 0.2 atmospheres. On the other hand, despite the careful experimental manipulation described by Warburg and Kubowitz, extra oxygen may have entered their system by diffusion out from the measuring column of Brodie's solution. It is possible that the discrepancy between the results of these experimenters and those of all others is to be attributed to the different cells used.

I am aware of only five sets of observations from which the entire  $C_s$ - $Q$  curve can be obtained. The older observations of Amberson (1928) on fertilized *Arbacia* eggs fit almost exactly with those of Tang (1931) on unfertilized ones, even the absolute  $C_0$  values agreeing. This is hardly to be expected with a  $Q_{O_2}$  five to six times greater for the fertilized than the resting eggs, and if this identity is confirmed when using the same method on both materials, it will be of considerable importance. Actually, the estimated  $Q_{O_2}$  for Amberson's fertilized eggs is less than Tang's value for unfertilized ones (see footnote to Table V), so little more can be said at this time. For bacteria, the curve obtained by Shoup (1929) is available. The complete  $Q$ - $C_s$  curve was not obtained in the case of *Sarcina* because of the continually changing  $Q_{O_2}$  even in air. Meyerhof (1916, 1917) has presented less complete data for the nitrifying bacteria. All

TABLE VI

| $\frac{C_s}{C_0}$ | $\frac{Q}{Q_0}$        |                      |                    |                    |
|-------------------|------------------------|----------------------|--------------------|--------------------|
|                   | <i>Arbacia</i><br>eggs | Luminous<br>bacteria | Nitrite<br>formers | Nitrate<br>formers |
| 1.00              | 1.00                   | 1.00                 | 1.00               | 1.00               |
| 0.90              | 0.99                   | 0.98                 | 0.99               | 0.98               |
| 0.65              | 0.98                   | 0.95                 | 0.97               | 0.88               |
| 0.50              | 0.96                   | 0.92                 | 0.92               | 0.80               |
| 0.35              | 0.92                   | 0.86                 | 0.79               | 0.71               |
| 0.22              | 0.85                   | 0.74                 | 0.54               | 0.59               |
| 0.10              | 0.66                   | 0.53                 | 0.24               | 0.34               |
| 0.04              | 0.46                   | 0.15                 | (.13)              |                    |
| 0.00              | 0.00                   | 0.00                 | 0.00               | 0.00               |

these data are summed up in Table VI. The oxygen consumed by each type of cell is expressed as a fraction of the maximum for that cell, and all are aligned against  $C_s$  values expressed as fractions of  $C_0$ , this being likewise taken as unity for all.

It is especially interesting to compare these observed curves with those calculated on the various types of assumption. For the luminous bacteria, the observed curve agrees fairly well with the one calculated on the assumption that there is no concentric zoning of the cell and that oxygen consumption is independent of pressure, except as diffusion becomes inadequate.  $Q$  falls with  $C_s$ , in other words, as would be expected if the only factor involved were the depth of penetration. Still the absolute value of  $C_0$  as measured is far above that expected on the grounds of the diffusion equation. Either, therefore, the diffusion coefficient for oxygen through the bacterial cell is over 1,000 times smaller than that for fascia and muscle,<sup>2</sup> or the fall of  $Q$  with  $C_s$  is quite independent of any diffusion factor.

In the case of sea urchin eggs, the situation is still more complicated. The observed curve does not fit the simple calculated one. It does fit, however, a curve calculated on the assumption that  $A$  is independent of  $C$  but including the complication that  $A$  is not uniform in all cell regions but is greater in a cortical zone than in the central portion (Curve I, Fig. 2, and Fig. 3). A lower diffusion constant in the cortical zone can not yield this shape of curve.

Again, the absolute value of  $C_c$ , 100 times smaller than that calculated, makes it very difficult to interpret the agreement with the curves calculated on a diffusion basis. If oxygen diffuses through sea urchin eggs 100 times more slowly than through muscle, all the results become quantitatively intelligible. But it is more probable that failing diffusion is only a very minor factor, even in these larger eggs, and that the direct fall of  $A$  with falling  $C$  is the primary one.

Assuming that  $A(C)$  is directly given by the  $Q(C)$  curve in the case of luminous bacteria and is obtained by correcting for diffusion in the case of *Arbacia* eggs, we are left with an empiric relation between oxygen concentration and rate of oxidations. Shoup (1929) offers a physical interpretation of his results in terms of adsorption of oxygen on or evaporation from surfaces in the cell. He puts Langmuir's (1916) equation for such gas adsorption in the form:

$$k_1 p(1 - \theta) = k_2 \theta$$

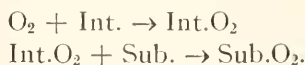
where  $k_1$  and  $k_2$  are adsorption and evaporation constants respectively and  $\theta$  is the fraction of the total surface covered, and indicates that this will fit his curve if  $\theta$  be assumed to represent the percentage of maximal respiration, *i.e.*,  $\frac{A}{A_0}$  ( $A_0$  being the rate of oxidation at oxygen

<sup>2</sup> Each bacterial cell, as any small particle, is surrounded by an envelope of fluid which is essentially not stirred. This increases the effective radius of the cell and hinders diffusion. This alone, however, to increase  $C_0$  1,000 times, would require a layer over 300 times the radius of the cell. (See Equation 7.)

pressure  $C_c$ ;  $A$  the rate at pressure  $C$ ). Warburg and Kubowitz (1929) also developed a relationship in terms of reaction velocities for oxidation and reduction of an iron catalyst.

A somewhat similar development follows.

It may be safely assumed that oxygen combines with at least one intermediate catalyst before acting upon the substrate. Then, neglecting any details,



For an equilibrium state, the concentration of  $\text{Int.O}_2$  must remain constant and the rates of its formation and reduction must be equal. So

$$[\text{O}_2][\text{Int.}]k_0 = A = [\text{Int.O}_2][\text{Sub.}]k_r, \quad (28)$$

where  $A$  is the velocity of combination of oxygen, or rate of respiration, and  $k_0$  and  $k_r$  constants of oxidation and reduction.

In the presence of an excess of substrate,  $[\text{Sub.}]$  may be assumed to be a constant,  $k_s$ , and

$$A = [\text{Int.O}_2]k_rk_s.$$

The maximal rate of respiration,  $A_0$ , will be obtained when all  $\text{Int.}$ ,  $\text{Int.}_t$ , is in the oxidized state; and the respiration as a fraction of the maximum is then

$$\frac{A}{A_0} = \frac{[\text{Int.O}_2]}{[\text{Int.}_t]}. \quad (29)$$

From (28)

$$\frac{[\text{Int.}]}{[\text{Int.O}_2]} = \frac{k_rk_s}{k_0[\text{O}_2]} = \frac{K}{[\text{O}_2]}.$$

Adding 1 to each side and inverting,

$$\frac{[\text{Int.O}_2]}{[\text{Int.}] + [\text{Int.O}_2]} = \frac{[\text{O}_2]}{K + [\text{O}_2]}.$$

But

$$[\text{Int.}] + [\text{Int.O}_2] = [\text{Int.}_t].$$

So that

$$\frac{A}{A_0} = \frac{[\text{O}_2]}{K + [\text{O}_2]}.^3 \quad (30)$$

<sup>3</sup> If  $[\text{Sub.}]$  is not assumed to be constant, the final equation is

$$\frac{A}{A_0} = \frac{[\text{O}_2]}{K[\text{Sub.}] + [\text{O}_2]}.$$

When  $[\text{Sub.}]$  is maximum, the effect of  $[\text{O}_2]$  is greatest; as  $[\text{Sub.}]$  becomes less, it, rather than  $[\text{O}_2]$ , becomes the critical factor.

But Langmuir's equation may be readily put in an identical form by using the notation  $[O_2]$  for  $p$ ,  $\frac{A}{A_0}$  for  $\theta$ , and  $K$  for  $\frac{k_2}{k_1}$ . Obviously, an agreement between the curve of this equation and that obtained experimentally fails to substantiate either of the assumed mechanisms as the one underlying these oxidations. As a matter of fact, the experimental data do not agree too well with the equation.

Shoup's data (as read from his curve) yield the following:

| Per cent $O_2$ | $\frac{A}{A_0}$ | $K$  |
|----------------|-----------------|------|
| 3.00 and over  | 1.00            | 0.00 |
| 1.32           | 0.90            | 0.14 |
| 0.79           | 0.80            | 0.20 |
| 0.55           | 0.70            | 0.23 |
| 0.38           | 0.60            | 0.25 |
| 0.27           | 0.50            | 0.27 |
| 0.22           | 0.40            | 0.33 |
| 0.17           | 0.30            | 0.40 |
| 0.13           | 0.20            | 0.52 |

It is sufficiently obvious that  $K$  is even roughly constant only in the middle range.

Tang's data, similarly handled, give:

| Per cent $O_2$ | $\frac{Q}{Q_0} \frac{A}{A_0}$ | $K$ |
|----------------|-------------------------------|-----|
| 10.0 and over  | 1.00                          | 0.0 |
| 9.0            | 0.99                          | 0.1 |
| 6.5            | 0.94                          | 0.3 |
| 5.0            | 0.87                          | 0.8 |
| 3.5            | 0.78                          | 1.0 |
| 2.2            | 0.66                          | 1.1 |
| 1.0            | 0.49                          | 1.0 |
| 0.4            | 0.27                          | 1.1 |
| 0.0            | 0.00                          | —   |

The agreement here is somewhat better.  $K$  is approximately constant up to 80 per cent maximal respiration.

For the nitrifying bacteria, the curves of the nitrite and the nitrate formers are widely different, and both are far from agreeing with the derived equation.

It seems to follow, clearly enough, that the  $Q-C_s$  curve is far from identical for the various cells, and that the individual form is probably but little dependent on the limits set by diffusion. No one

| NO <sub>2</sub>         |                 |     | NO <sub>3</sub> |     |
|-------------------------|-----------------|-----|-----------------|-----|
| Per cent O <sub>2</sub> | $\frac{Q}{Q_0}$ | $K$ | $\frac{Q}{Q_0}$ | $K$ |
| 20 and over             | 1.00            | 0.0 | 1.00            | 0.0 |
| 15                      | 0.99 +          | 0.1 | 0.92            | 1.3 |
| 10                      | 0.98            | 0.2 | 0.80            | 2.5 |
| 7                       | 0.92            | 0.7 | 0.72            | 7.0 |
| 4                       | 0.68            | 1.9 | 0.57            | 4.0 |
| 2                       | 0.35            | 3.7 | 0.34            | 3.9 |

simple assumption as to oxidizing mechanism appears able to fit all cases. Additional data for a much greater variety of cells are greatly to be desired, and must precede any more elaborate attempt to theoretically derive the important  $A(C)$  curve.

Further important questions concern the change in  $Q_0$  attending changed conditions. The fertilized sea urchin egg, for example, after a tremendous initial burst of oxidation, continues to use oxygen at five to six times the resting rate. Is this to be interpreted in terms of a sudden increase of available Int.? None the less, so far as present data show, there is no change in  $C_0$  or in  $A(C)$ . *Sarcina*, on the other hand, shows an increase in  $C_0$  approximately in proportion to the increased  $Q$  when glucose is added. The complicated relations between lactic acid and  $Q_0$  have been previously discussed (Gerard, 1931). The suddenly increased respiration of muscle, nerve, and other cells associated with activity also comes to mind in this connection.

Finally, it is to be noted that most cells exhibit a considerable "factor of safety" in their rate of energy liberation. The respiration may be very considerably depressed by low oxygen pressures before the lessened energy interferes with the physiological activity of a cell. Thus luminous bacteria do not begin to dim until the oxygen is reduced to 0.25 per cent (Shoup, 1929), when respiration has been reduced to half, and still luminesce at 0.0007 per cent O<sub>2</sub> (Harvey, 1928). *Arbacia* eggs show no disturbance in cleavage at oxygen concentrations over 1.5 per cent (Amberson, 1928) though 10 per cent is required for maximal respiration.

Similar relations are difficult to demonstrate for tissues because of the tremendously greater importance of diffusion in the larger masses. For the case of nerve, it was shown (Gerard, 1927) that the  $C_0$  calculated from the simple diffusion equation, assuming  $A$  independent of  $C$ , was in good agreement with the observed value. It is probable, however, that more careful examination of this point would reveal



some dependence of  $A$  on  $C$ . Less direct evidence indicates that here, also, conduction is possible when the energy available is but a fraction of the normal amount. (Gerard, 1930).

#### SUMMARY

The question of diffusion of oxygen into cells, in distinction to tissues, requires examination of several factors not previously considered. The relation between external oxygen pressure and rate of respiration may be complicated by the existence of different diffusion constants and respiration rates in separate cell zones.

Equations are developed covering such cases for two concentric zones in a sphere and relating rate of respiration to oxygen concentration, cell radius, and diffusion coefficient.

When the assumption, made in similar studies for tissues, that consumption in any one region is independent of oxygen pressure if above zero, is avoided, the observed consumption-pressure curve can be corrected for diffusion effects to give the true consumption-pressure relation. Equations for this correction are presented.

Available data on bacteria, marine eggs, and other material, are examined quantitatively. It appears very probable that oxygen consumption in each region is dependent on oxygen concentration up to quite considerable values. The theoretical bases for such a dependence are discussed.

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